

Title: Cloning LuxI-GFP

Goal: To get LuxI in front of GFP

Date range: July-August 2015

Members that worked on project: Michael Donovan

Summary: Due to contamination of Distribution Kit LuxI the construct is not on 1C3

New part number: BBa_K1718004

Part size: 1550

Transformation:

- Pulled BBa_C0061 (LuxI on 1C3) and BBa_I13504 (GFP on 1A2) from iGEM kit.
- 1 ul of plasmid from iGEM kit into 40ul of 5 alpha competent cells.
- Let cells sit with plasmid on ice for 30 minutes.
- Heat shock at 42°C for 45 seconds.
- Put on ice for 5 minutes.
- Treat cells with 950ul of SOC.
- Put in incubator at 37°C for 1 to 2 hours.
- Spread 100ul of cells onto chloramphenicol resistance plates (170ng/ul concentration) or ampicillin plates (100 ng/ul)

Overnight:

- One colony from each plate into 5 mL of LB, 25ul chloramphenicol or 5 ul ampicillin

Mini-prep:

- GFP 1: 358 ng/ul GFP 2: 110 ng/ul LuxI 1:258 ng/ul LuxI 2: 208 ng/ul

Digestion:

- For ligation
- Digestion of:BBa_C0061

Reagent	10 ul reaction	Concentration
Cutsmart Buffer	1 ul	1 x
SpeI	.2 ul	2 units
PstI-HF	.2 ul	2 units
DNA (258ng/ul)	.5 ul	129.5 ng
Water	8.1 ul	

- Digestion of: BBa_I13504

Reagent	30 ul reaction	Concentration
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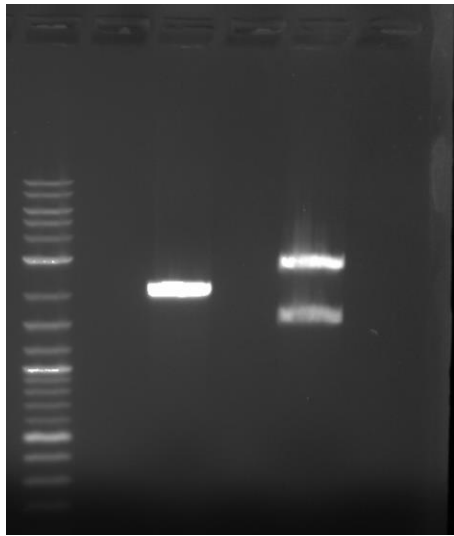
Cutsmart Buffer	3 ul	1 x
Xbal	.6 ul	6 units
PstI-HF	.6 ul	6 units
DNA (110 ng/ul)	3 ul	330 ng
Water	22.8 ul	

Ligation:

- BBa_C0061 S+P and BBa_I13504 X+P
- Volume (and concentration if known) of parts

Component	20 ul reaction	Concentration/amount
10XT4 DNA Ligase Buffer	2 ul	1 x
Vector DNA (C0061)	1 ul	
Insert DNA (I13504)	11 ul	
T4 DNA Ligase	1 ul	
Nuclease-free water	5 ul	

Transformed ligation product using same protocol as before.



Lane 1: 2-log ladder

Lane 5: LuxI+GFP on contamination backbone

Expected length~1500

Colonies on plates green due to contamination of LuxI, same as in LuxI+RFP.
Next step, add behind gate.

Sequence:

- Primers used: VF2 and VR
- Result: 100% match

Part submission:

- BBa_K1718004